## -25-of-28-

1	WHAT I	SCL	AIMED	IS

- 1 1. A method for the vitrification of biological materials, said method comprising the
- 2 steps of:

7

- 3 (a) suspending the biological material in a cryoprotective equilibration medium,
- 4 having a concentration of cryoprotectant(s) below that sufficient to protect against
- 5 ice formation to the glass transition temperature of the cryoprotective equilibration
- 6 medium;
  - (b) rinsing the equilibrated biological material with a vitrification solution, the
- 8 vitrification medium having a concentration of cryoprotectant(s) sufficient to
- 9 protect against ice formation to the glass transition temperature of the vitrification
- 10 medium; and
- 11 (c) dropping the vitrification solution-rinsed biological material in microdroplets
- of vitrification solution onto a solid surface with good heat conductivity having
- been previously cooled down to about -150°C to about -180°C.
  - 1 2. The method of claim 1 wherein the biological material is a cell.
  - 1 3. The method of claim 1 wherein the biological material is an oocyte.
  - 1 4. The method of claim 1 wherein the biological material is an embryo.
  - 1 5. An improved method for cryopreserving biological material suspended in a
  - 2 vitrification solution, wherein the improvement comprises contacting microdroplets of the
  - 3 vitrification solution containing the biological material with a solid surface having a
  - 4 temperature of about -150°C to about -180°C, said surface having a good heat
  - 5 conductivity.
  - 1 6. The method of claim 5 wherein the biological material is a cell.
  - 1 7. The method of claim 5 wherein the biological material is an oocyte.

## -26-of-28-

- 1 8. The method of claim 5 wherein the biological material is an embryo.
- 1 9. An improved method for cryopreserving biological material suspended in a
- 2 vitrification solution, wherein the improvement comprises contacting microdroplets of the
- 3 vitrification solution containing the biological material with a solid surface having a
- 4 temperature of about -150°C to about -180°C, said surface having a thermal conductivity at
- 5 20°C of greater than about 10 W/(m-k).
- 1 10. A method for the vitrification of oocytes, said method comprising the steps of:
- 2 (a) suspending the oocytes in a cryoprotective equilibration medium, having a
- concentration of cryoprotectant(s) below that sufficient to protect against ice
  formation to the glass transition temperature of the cryoprotective equilibration
- formation to the glass transition temperature of the cryoprotective equilibrat
- 5 medium;
- (b) rinsing the equilibrated oocytes with a vitrification solution, the vitrification
   medium having a concentration of cryoprotectant(s) sufficient to protect against ice
- formation to the glass transition temperature of the vitrification medium; and
- 9 (c) dropping the vitrification solution-rinsed occytes in microdroplets of
- 9 (c) dropping the vitrification solution-rinsed occytes in microdroplets of vitrification solution onto a solid surface with good heat conductivity having been
- previous cooled down to about -150°C to about -180°C.
- 1 11. An improved method of transferring nuclear DNA from a donor cell to an
- 2 enucleated oocyte, said improvement comprising the step of introducing the nuclear
- 3 material of the donor cell into an enucleated oocyte derived from an oocyte vitrified by the
- 4 method of claim 10.
- 1 12. An oocyte vitrified by the method of claim 10.
- 13. An embryo developed from the oocyte of claim 12.
- A fetus developed from the oocyte of claim 12.
- An animal developed from the oocyte of claim 12.

## -27-of-28-

- 1 16. A cell line developed from the embryo of claim 13.
- 1 17. A cell line developed from the fetus of claim 14.
- 1 18. A cell line developed from the animal of claim 15.
- 1 19. An improved method for cryopreserving oocytes suspended in a vitrification
- 2 solution, wherein the improvement comprises contacting microdroplets of the vitrification
- 3 solution containing the oocytes with a solid surface having a temperature of about -150°C
- 4 to about -180°C, said surface having a thermal conductivity 20°C of greater than about 10
- 5 W/(m-k).

1

- 1 20. An oocyte vitrified by the method of claim 19.
- 1 21. An embryo developed from the oocyte of claim 20.
- 1 22. A fetus developed from the oocyte of claim 20.
- I 23. An animal developed from the oocyte of claim 20.
  - A cell line developed from the embryo of claim 21.
- 1 25. A cell line developed from the fetus of claim 22.
- 1 26. A cell line developed from the animal of claim 23.
- 1 27. An improved method for the parthenogenetic development of vitrified oocytes
- 2 cultured in a KSOM plus BSA culture system, the improvement comprising co-culturing
- 3 with cumulus-cells.